

# Karyotype and chromosomal polymorphism of *Chironomus luridus* Strenzke, 1959 (Diptera: Chironomidae) in European and Asian populations

I.I. Kiknadze, A.G. Istomina

Institute of Cytology and Genetics, SB RAS, Prosp. Akademika Lavrentieva 10, Novosibirsk, 630090 Russia.  
E-mail: kiknadze@bionet.nsc.ru

**Abstract.** The karyotype structure and chromosomal polymorphism were studied in several European (European part of Russia and the Netherlands) and Asian (Siberia and Kazakhstan) populations of *Chironomus luridus* Strenzke, 1959. Inversion polymorphism was detected in six of the seven chromosome arms: three banding sequences detected in arm A, six sequences in arm B, two sequences in arm C, three sequences in arm E, five sequences in arm F, and two sequences in arm G. Only arm D was monomorphic in all studied populations. In total, 22 banding sequences were recorded in *Ch. luridus*; they form the banding sequence pool of this species. Thus, *Ch. luridus* can be regarded as a very polymorphic species. However, the European and Asian populations differed considerably in the levels of polymorphism: the Asian populations were less polymorphic, containing only 8 to 10 sequences, whereas the European populations had 11 to 16 sequences. The new banding sequences lurA3, lurB5, lurE3 were found in Asian populations, whereas the sequences lurB2, lurB3, lurB4, lurB6, lurE2, lurF1, lurF2a, lurF3, lurF4, and lurG2 are lacked. The total level of inversion heterozygosity in the Asian populations was 12-25% versus 78-80% in the European populations.

**Key words:** karyotype, banding sequences, chromosomal polymorphism, *Chironomus luridus*.

## INTRODUCTION

Inversions play a key role in the evolution of animal karyotypes. They change the normal order of genes within a chromosome, which has important consequences in the evolution of a species. Molecular analysis of genomes and proteomes has confirmed that the genomes of distant species, such as man, domestic mouse, *Drosophila* Fallén, 1823, and *Anopheles* Meigen, 1818 differ mainly by the order of genes in chromosomes (linkage group) rather than the number and the set of genes (Zdobnov et al., 2002; Ayala,

Coluzzi, 2005). The contributions of other chromosome rearrangements, in particular, reciprocal whole-arm translocations, are more limited (White, 1973; King, 1993). However, for the genus *Chironomus* Meigen, 1803, reciprocal whole-arm translocations proved to be the main rearrangements that have led to the formation of the so-called cytocomplexes of species, differing in the combinations of seven chromosome arms (Keyl, 1962; Martin, 1979, 2007; Wülker, 1980, 2007). In particular, the karyotypes of the species belonging to the “thummi” cytocomplex have the chromosome arm combination AB CD

**Table 1.** Collection sites and number of *Chironomus luridus* larvae analyzed.

Location	Population	Collection data	Number of larvae analyzed
Russia			
Yaroslavl Prov., Borok, Latka river	RU-YAR-LA	30.06.1986 21.05.1987 15.09.1989	104
Novosibirsk, Eltsovka river	RU-NSK-EL	16.05.2001	50
Novosibirsk, the basin of Eltsovka river, gard. com. "Kristall"	RU-NSK-KR	22.09.2002	19
Novosibirsk, Ziryanka river	RU-NSK-ZI	05.05.2008	8
Kazakhstan, Semipalatinsk nuclear polygon			
Uzun-Bulak river, Degelen	KZ-SIP-UB	21.06.2000	79
Creek of Shagan River	KZ-SIP-SH	24.06.2000	4
The Netherlands, Germany and Belgium			
Gemert, Molenbroekse Loop, Bovenslinge, Strijper Aa, Leegmoor, nature reserve Waelenhoek (Niel) clay pit (Niel 8)	NL-DE-BE	22.07.2008	3
		15.05.2007	2
		11.09.2007	2
		02.05.2008	1
		09.03.1994	1

EF G in the four chromosomes of the haploid set; “pseudothummi” cytocomplex - AE CD BF G; “camptochironomus” cytocomplex - AB CF ED G; “parathummi” cytocomplex - AC ED BF G; “maturus” cytocomplex - AF CD EB G; and so on. The “thummi” and “pseudothummi” cytocomplexes contain the most species. The karyotype structure and chromosomal polymorphism have been so far comprehensively studied only in the “thummi” cytocomplex. The species of the “pseudothummi” cytocomplex still require further study. In addition, the effect of reciprocal whole-arm translocations on the structure of the centromeric regions in AE and BF translocated chromosomes has been recently demonstrated in *Ch. dorsalis* Meigen, 1818, a member of the “pseudothummi” cytocomplex. The translocated chromosomes

became dicentric (Kiknadze et al., 2008b). A loss of one of the centromeres and appearance of a neocentromere has been suggested in the translocated chromosome AE in another species of this cytocomplex, *Ch. saxatilis* Wülker, Ryser et Scholl, 1981 (Shobanov, Petrova, 1995). It is still unclear whether such phenomena are characteristic of only some members of the “pseudothummi” cytocomplex or they are common to all the species of this cytocomplex. In addition, it has been demonstrated that the changes in chromosome arm combinations (linkage groups combinations in Diptera) result in emergence of new inversion breakpoints stimulating divergence of cytocomplexes during evolution (Kiknadze et al., 2003). In this work, we have studied in detail the karyotype of *Ch. luridus*, a member of the

**Table 2.** Frequencies of banding sequences in natural populations of *Chironomus luridus*. \*Keyl’s data (1962), frequencies of banding sequences were not determined; presence of banding sequence is marked by +. N – the number of individuals.

Banding sequences	Populations							
	West Europe		East Europe	West Siberia, Novosibirsk Province			Kazakhstan, Semipalatinsk nuclear polygon	
	NL-DE-BE N=9	Germany*	RU-YAR-LA N=104	RU- NSK-EL N=50	RU- NSK-KR N=19	RU- NSK-ZI N=8	KZ-SIP-UB N=79	KZ-SIP-SH N=4
lurA1	1.000	+	0.962	0.990	1.000	1.000	1.000	1.000
lurA2	0	+	0.038	0	0	0	0	0
lurA3	0	0	0	0.010	0	0	0	0
lurB1	0.889	+	0.971	1.000	1.000	0.938	1.000	1.000
lurB2	0	0	0.019	0	0	0	0	0
lurB3	0.056	0	0.005	0	0	0	0	0
lurB4	0	0	0.005	0	0	0	0	0
lurB5	0	0	0	0	0	0.062	0	0
lurB6	0.056	0	0	0	0	0	0	0
lurC1	0.500	+	0.716	0.960	0.947	1.000	0.899	0.875
lurC2	0.500	0	0.284	0.040	0.053	0	0.101	0.125
lurD1	1.000	+	1.000	1.000	1.000	1.000	1.000	1.000
lurE1	1.000	+	0.995	0.990	1.000	1.000	1.000	1.000
lurE2	0	0	0.005	0	0	0	0	0
lurE3	0	0	0	0.010	0	0	0	0
lurF1	0.111	+	0	0	0	0	0	0
lurF2	0.556	+	0.538	1.000	1.000	1.000	1.000	1.000
lurF2a	0	+	0.375	0	0	0	0	0
lurF3	0.333	+	0	0	0	0	0	0
lurF4	0	0	0.087	0	0	0	0	0
lurG1	0.889	+	0.995	1.000	1.000	1.000	1.000	1.000
lurG2	0.111	0	0.005	0	0	0	0	0

“pseudothummi” cytocomplex. Earlier the karyotype of *Ch. luridus* was described by Keyl and Keyl (1959) with mapping of arms A, E, and F (Keyl, 1962). Additional information about the *Ch. luridus* karyotype has been reported by Belyanina (1983), Kiknadze et al. (1988, 1991) and Michailova (1989). The chromosome polymorphism in arms A, E, and F in German populations was briefly described by Keyl (1962). A high level of chromosome polymorphism in Western European *Ch. luridus* populations was noted by Acton (1957), although this author

erroneously identified this species as *Ch. dorsalis*. We have evaluated quantitatively the chromosome polymorphism in several European and Asian populations. We were the first to map chromosome arms C and D and discover inversion polymorphisms in six of the seven chromosome arms (arms A, B, C, E, F, and G). Considerable differences between the levels of chromosome polymorphism of the European and Asian populations studied were found. Structural changes in the centromeric regions on translocated chromosomes were studied.

## MATERIAL AND METHODS

*Ch. luridus* larvae of the last (fourth) instar were used in the work. The collection sites and sample sizes are listed in Table 1. The larvae were fixed in a mixture of 96% ethanol and glacial acetic acid (3:1) and stored in a refrigerator. Squash preparations of the salivary gland polytene chromosomes were made conventionally, using aceto-orcein staining (Kiknadze et al., 1991). Polytene chromosome arms A, E, and F were mapped according to Keyl (1962) and arms C and D, according to Dévai et al. (1989), using the banding sequences of *Chironomus piger* Strenzke, 1959 polytene chromosomes as a standard. Arms B and G were not mapped due to complex chromosome rearrangements, as compared with *Ch. piger*. Inversion banding sequences of polytene chromosomes were designated using the abbreviated species name, arm designation, and banding sequence number (lurA1, lurA2, lurA3, lurD1, lurB2 etc.). Genotype combinations of banding sequences were designated as lurA1.1, lurB1.1, lurC1.1 etc., for homozygotes and as lurA1.2, lurB1.2, and lurB1.3, for heterozygotes.

The following cytogenetic characteristics of chromosomal polymorphisms in populations were used: the set, number and frequency of banding sequences and their genotypic combinations, the percent of the larvae with heterozygous inversions, and the mean number of heterozygous inversions per individual.

The studied cytological slides and fixed larval body after dissection of salivary glands are preserved in the collection of the Institute of Cytology and Genetics of Russian Academy of Sciences, Novosibirsk, Russia.

Equipment of the Center of Microscopy Analysis of Biological Objects of SB RAS in the Institute of Cytology and Genetics (Novosibirsk) was used in this work: microscope

“Axioskop” 2 Plus, CCD camera AxioCam HRc, software package AxioVision 4 (Zeiss, Germany).

## RESULTS

### Karyotype

The karyotype of *Ch. luridus* (Fig. 1) has a haploid number  $n=4$  with the chromosome arm combinations AE CD BF G (the “pseudothummi” cytocomplex). Chromosomes CD and BF are metacentrics; AE, submetacentric; and G, telocentric. The nucleolus is single and is localized on arm G near the centromeric–telomeric end. The karyotype contains four Balbiani rings: three in arm G and one in arm B (Fig. 1). The karyotype of *Ch. luridus* from different populations studied was identical with standard, described by Keyl, Keyl (1959) and Keyl (1962).

### Banding sequences

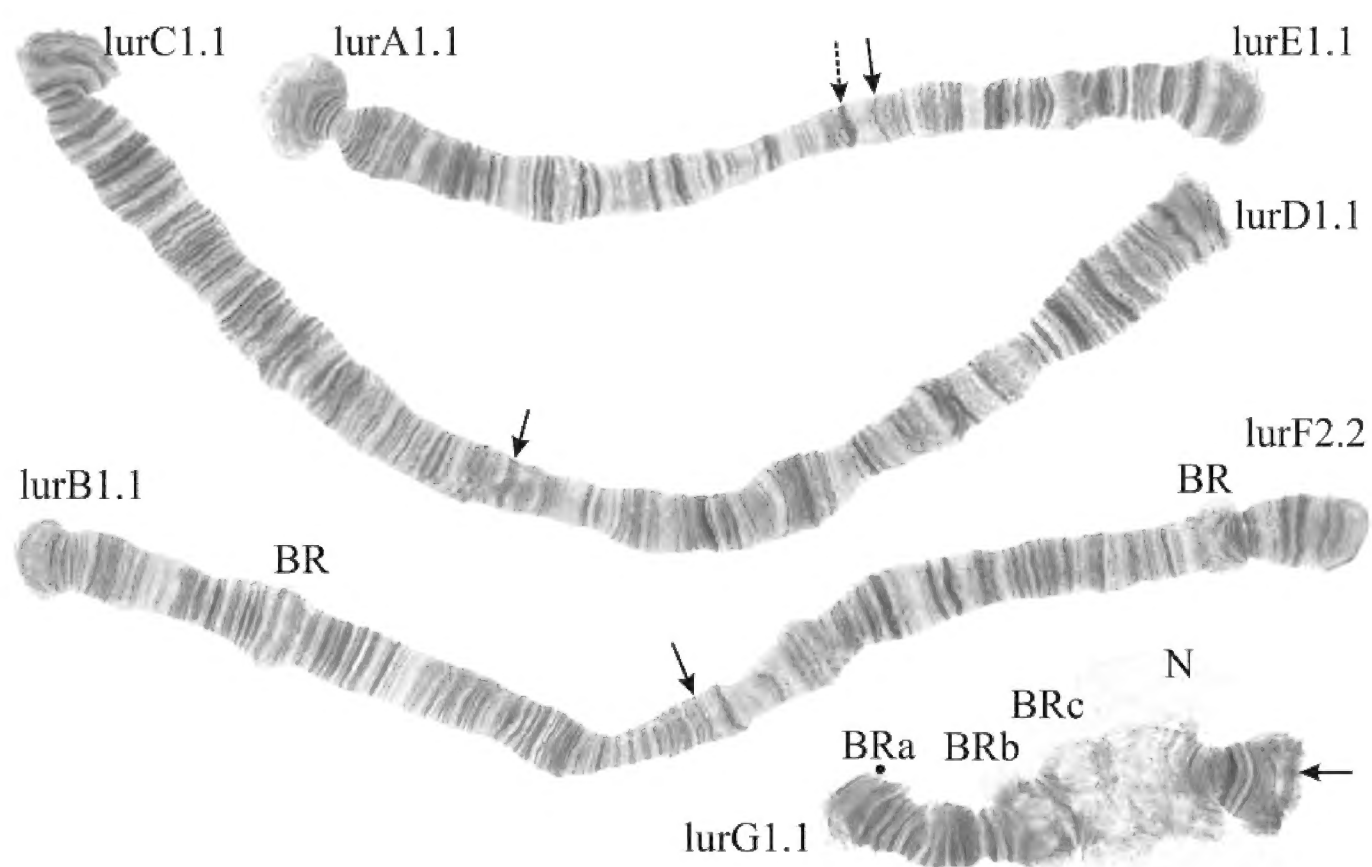
#### East European (Yaroslavl) population

**Arm A** is polymorphic and occurs in two banding sequences, lurA1 and lurA2, differing by a simple paracentric inversion (Tables 2-3; Figs 2, a; 3, a). The sequence lurA1 is identical to dorA1 in *Ch. dorsalis* and differs from the standard sequence pigA1 by only three overlapping inversions:

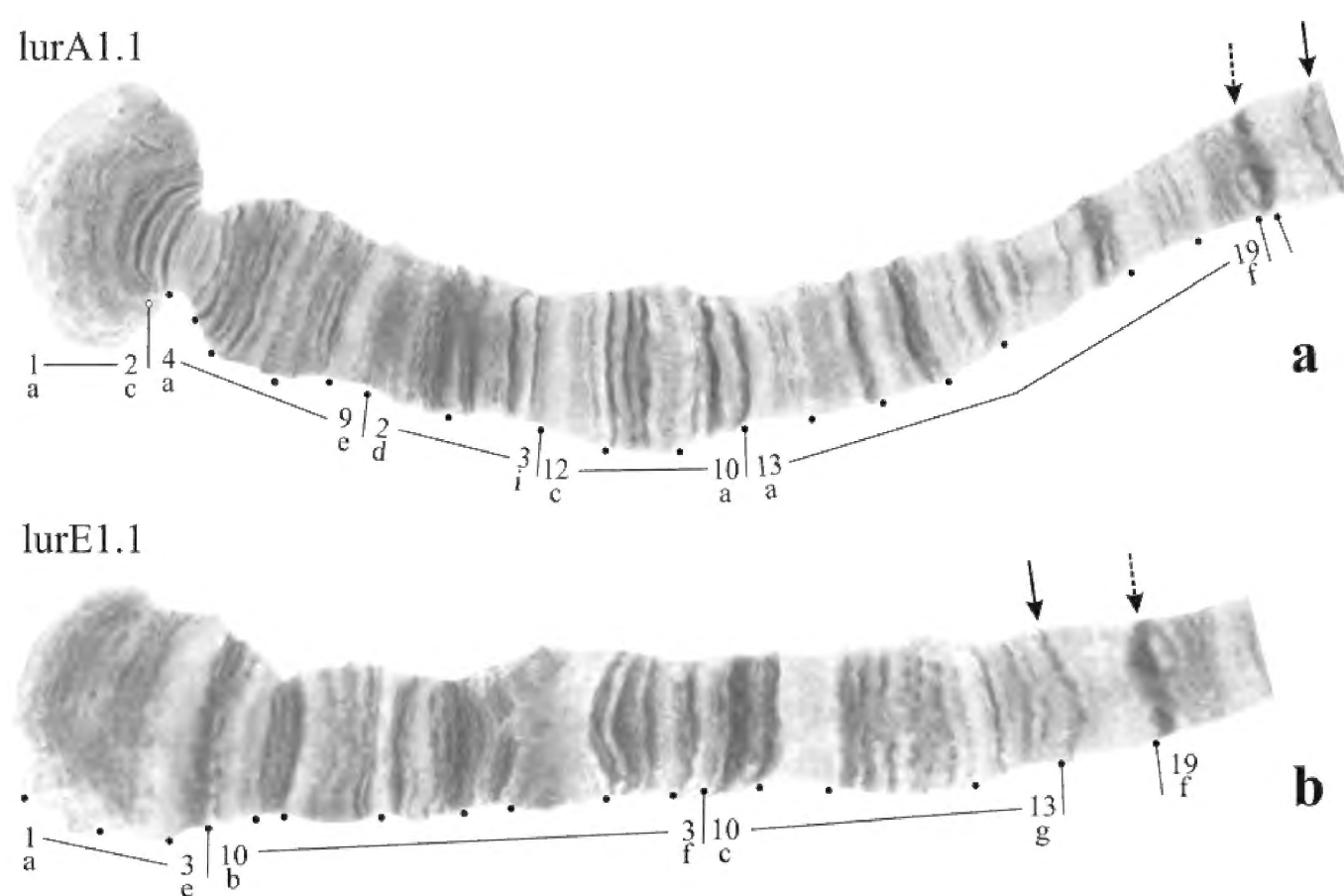
pigA1	1a-2c	<u>2d-3i</u>	<u>4a-9e</u>	<u>10a-12c</u>	13a-19f
Hyp	1a-2c	<u>12c-10a</u>	<u>9e-4a</u>	<u>3i-2d</u>	13a-19f
Hyp	1a-2c	<u>4a-9e</u>	<u>10a-12c</u>	<u>3i-2d</u>	13a-19f
lurA1=	1a-2c	<u>4a-6e</u>	<u>7a-9e</u>	<u>2d-3i</u>	<u>12c-10a</u> 13a-19f
dorA1					
lurA2	1a-2c	4a-6e	3i-2d	9e-7a	12c-10a 13a-19f

The sequence lurA1 is predominant in the populations studied, whereas the sequence lurA2 is rare and observed only as heterozygotes (Tables 2-3). The additional sequence lurA3 formed by short pericentric inversion was found in Novosibirsk populations.

**Arm E** is polymorphic and has two banding



**Fig. 1.** Karyotype of *Chironomus luridus*. lurA1.1, lurB1.1, etc., are the designations of genotypic combinations of banding sequences in chromosome arms; N – nucleolus; BR – Balbiani ring. Solid arrows show the centromeric bands, and dashed arrows indicate bands 19ef on arm A, which demonstrates neocentromeric character in *Ch. dorsalis*.



**Fig. 2, a, b.** Banding sequences in the arms A and E of *Chironomus luridus*. **a** - lurA1.1. **b** - lurE1.1. The designations are the same as in Fig. 1.

sequences, lurE1 and lurE2, differing by one simple inversion (Tables 2-3; Figs 2, b; 3, b). The sequence lurE1 is predominant, whereas lurE2 is very rare and has been detected only as heterozygotes. The former sequence is close to pigE1 and differs from it only by a simple inversion:

pigE1	1a-3e	<u>3f-10b</u>	10c-13g
lurE1	1a-3e	<u>10b-5a</u>	4h-3f 10c-13g
lurE2	1a-3e	<u>5a-10b</u>	4h-3f 10c-13g

The sequence lurE1 is identical with many *Chironomus* species (basic sequence).

Characteristic of lurE1 is a loosened state of region 5 (dark puff), which suggests a transcriptional activity of this region. The additional sequence lurE3 formed by short pericentric inversion was found in Novosibirsk populations.

**Arm C** is polymorphic and occurs in two banding sequences, lurC1 and lurC2, differing by one large simple inversion (Tables 2-3; Figs 2, c-d; 3, d). The sequence lurC1 differs from the standard pigC1 by three included inversions in the distal part of the arm. The proximal part (regions 9a-22) is completely identical to pigC1:

pigC1	1a-h	<u>1i-6h</u>	7a-8g	9a-22g
Hyp	1a-h	<u>6h-1i</u>	7a-8g	9a-22g
Hyp	1a-h	<u>8g-7a</u>	<u>1i-5c</u>	5d-6h 9a-22g
lurC1	1a-h	<u>5c-1i</u>	<u>7a-8c</u>	<u>8d-g</u> 5d-6h 9a-16d 16e-22g
lurC2	1a-h	5c-1i	7a-8c	<u>16d-9a</u> <u>6h-5d</u> 8g-d 16e-22g

The sequence lurC1 is dominant, but lurC2 is still rather frequent (Table 2), mainly as heterozygotes (Table 3).

**Arm D** is monomorphic (Tables 2-3; Fig. 2, e). The sequence lurD1 is considerably changed as compared with the standard pigD1 by five included inversions:

pigD1	1a-g	<u>1h-19f</u>	19g-24g
Hyp	1a-g	<u>19f-12c</u>	<u>12b-1h</u> 19g-24g
Hyp	1a-g	<u>12c-19f</u>	<u>1h-10c</u> <u>10d-12b</u> 19g-24g
lurD1	1a-g	12c-19f	<u>10c-1h</u> <u>12b-10d</u> 19g-24g

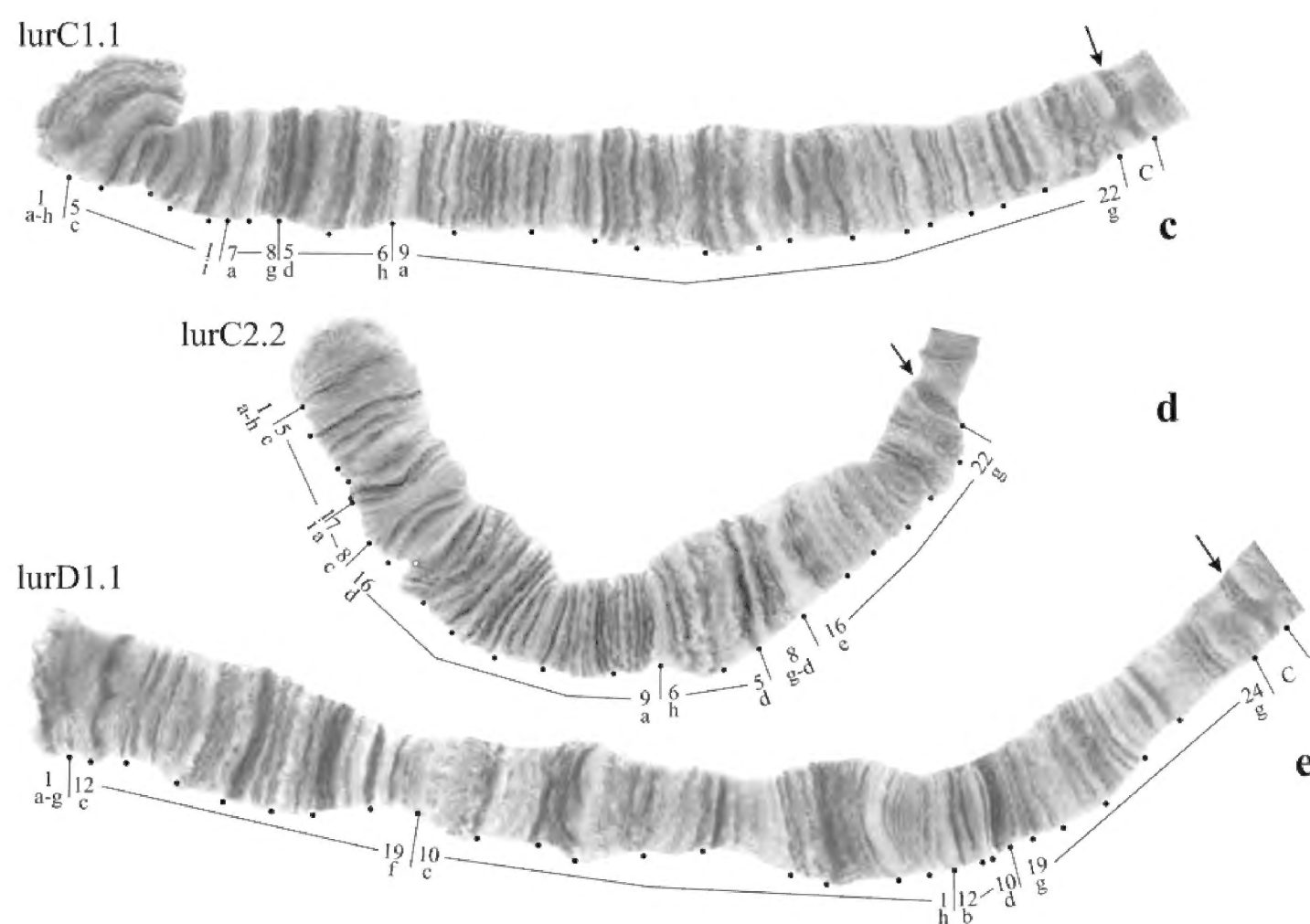
**Arm B** is polymorphic and occurs in four banding sequences - lurB1, lurB2, lurB3, and lurB4 (Tables 2-3; Figs 2, f; 3, e-i). The sequence lurB1 is dominant, while the remaining sequences are met rarely and only as heterozygotes (Tables 2-3).

The banding sequences in arm B have not been mapped. The localization of heterozygous inversions is shown in Figs 2, f; 3, e-i. The inversion in lurB2 sequence covers the central part of the arm (Figs 2, f; 3, e); the inversion in lurB3 sequence is located at the end of the arm (Figs 2, f; 3, f); and the inversion in lurB4 is small and located at the proximal part of the arm (Figs 2, f; 3, g).

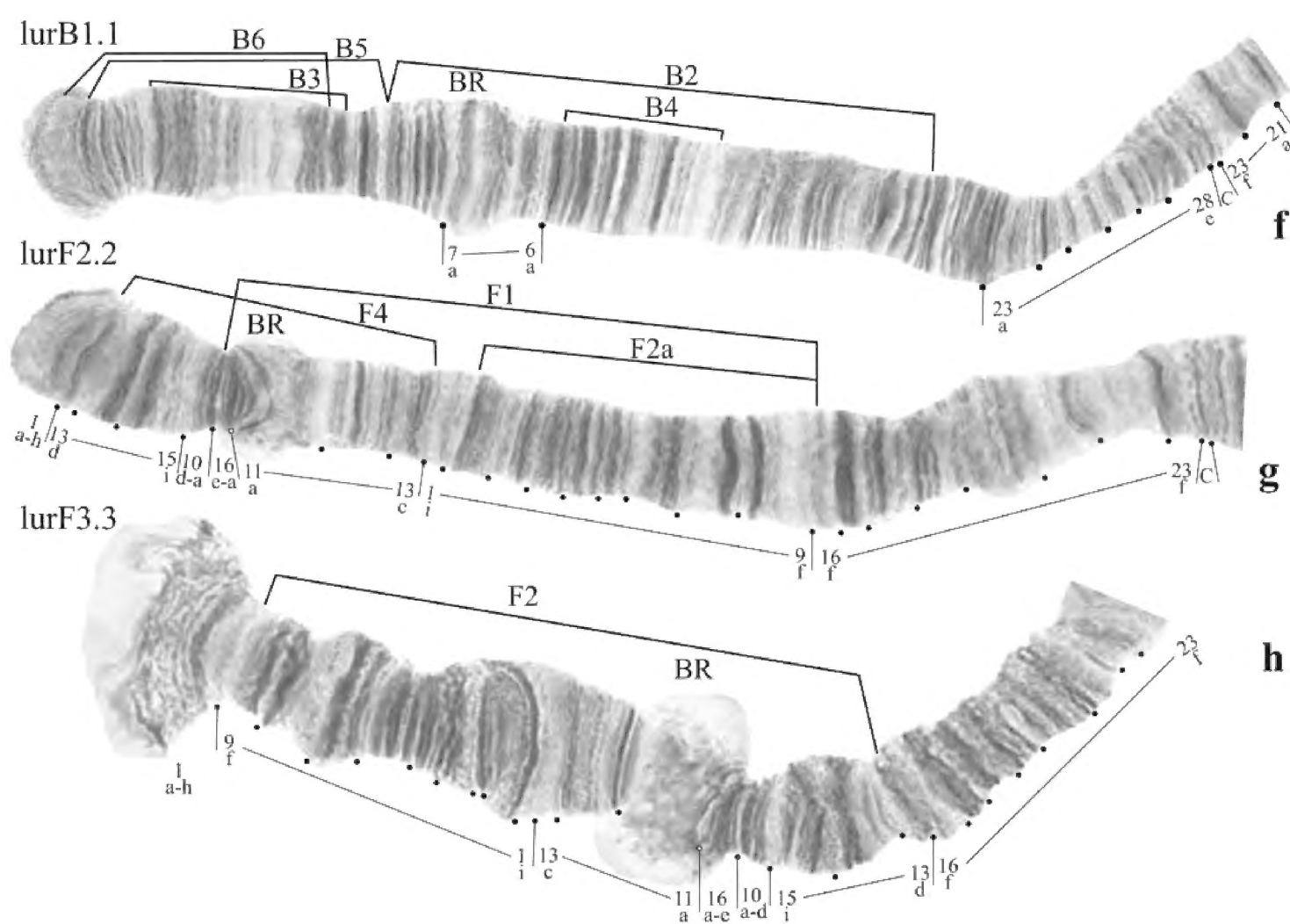
**Arm F** is polymorphic (Tables 2-3; Figs 2, g-h; 3, k-m). Of the four sequences described by Keyl (1962) for arm F in German populations (lurFI, lurFII, lurFIIa, and lurFIII), we found two sequences, lurF2 and lurF2a, and discovered a new sequence, lurF4. The sequence lurF1, which is characteristic of German populations, was not detected in the Yaroslavl population. Evolution of the banding sequences in arm F is connected with simple inversions. The sequence lurF1 differs from the standard pigF1 by two overlapping inversions; the inversion sequences intermediate between pigF1 and lurF1 were found in *Ch. holomelas* Keyl, 1961 (holF1) and *Ch. dorsalis* (dorF1):

pigF1	1a-10d	<u>11a-15i</u>	16a-23f
holF1=	1a-h	<u>1i-10d</u>	<u>15i-13d</u> 13c-11a 16a-23f
dorF1			
lurF1	1a-h	<u>13d-15i</u>	10d-a <u>9f-1i</u> 13c-11a 16a-e 16f-23f
lurF2	1a-h	<u>13d-15i</u>	10d-a <u>16e-a</u> <u>11a-13c</u> 1i-9f 16f-23f
lurF4	1a-h	13c-11a	16a-e 10a-d 15i-13d 1i-2a <u>2b-9f</u> 16f-23f
lurF2a	1a-h	13d-15i	10d-a 16e-a 11a-13c 1i-2a 9f-2b 16f-23f

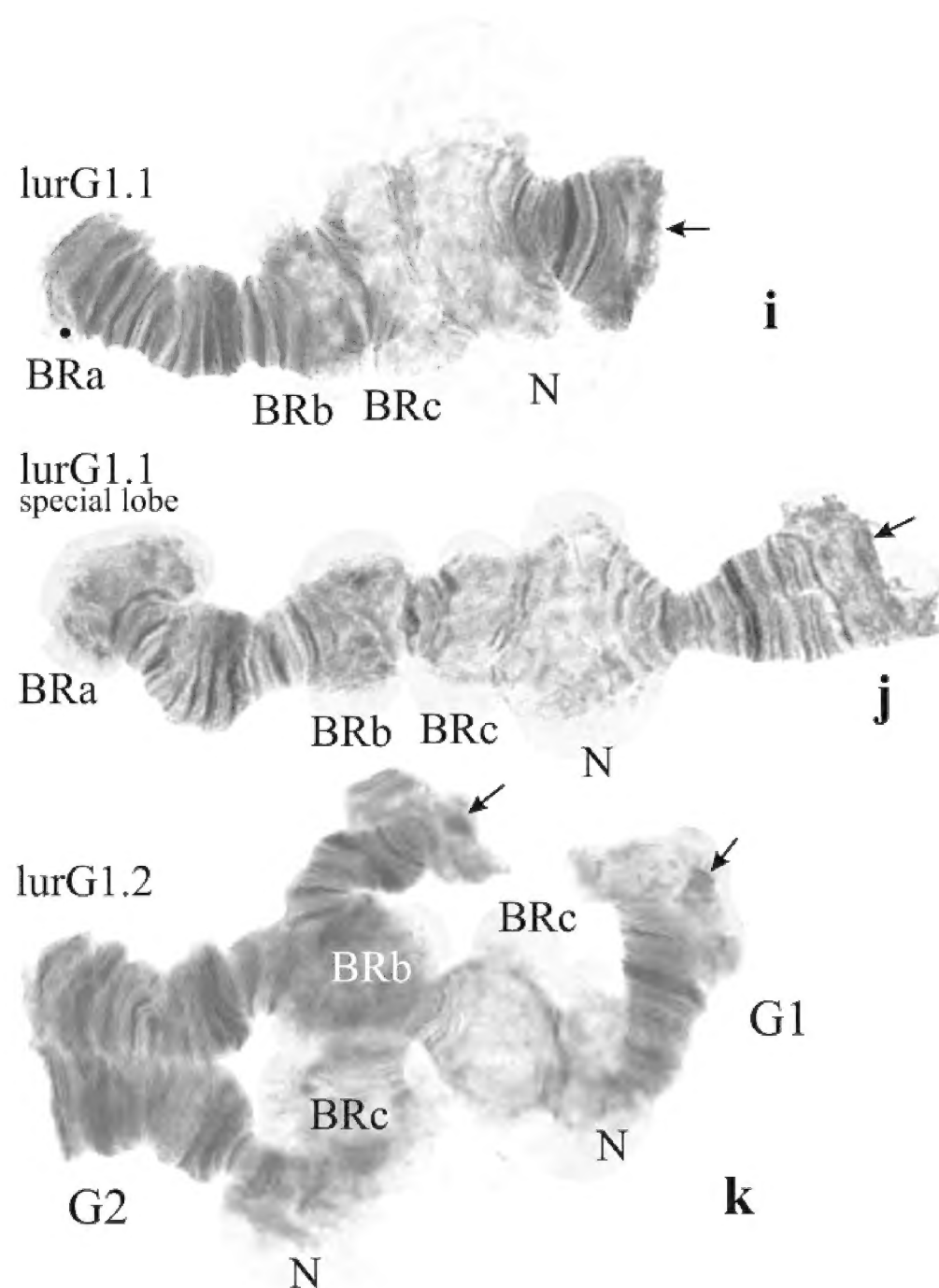
The sequence lurF2a was formed by simple inversion 9f-2b from lurF2. The sequence lurF3 earlier recorded by Keyl (1962) in German populations was not found in the Yaroslavl population, but it is present in the Netherlands population.



**Fig. 2, c-e.** Banding sequences in the arms C and D of *Chironomus luridus*. **c** - lurC1.1. **d** - lurC2.2. **e** - lurD1.1. The designations are the same as in Fig. 1.



**Fig. 2, f-h.** Banding sequences in the arms B and F of *Chironomus luridus*. **f** - lurB1.1. **g** - lurF2.2. **h** - lurF3.3. Brackets above arms indicate the localization of inversions. The designations are the same as in Fig. 1.



**Fig. 2, i, k.** Arm G in *Chironomus luridus*. **i** - lurG1.1 from the cells of salivary gland main lobe. **j** - lurG1.1 from the cells of salivary gland special lobe. **k** - heterozygote lurG1.2. BRa, BRb, and BRc are Balbiani rings a, b, and c. The rest designations are the same as in Fig. 1.

Only lurF2 and lurF2a have been detected as homozygotes and heterozygotes, whereas lurF4 was found only as heterozygotes (Table 3).

**Arm G** is weakly polymorphic with a predominance of lurG1 (Tables 2-3; Figs 2, i-k). Only one larva displayed the heterozygote lurG1.2. Arm G in *Ch. luridus* is very similar in the localization and molecular characteristics of transcriptionally active loci - nucleolus and Balbiani rings - with arm G in the standard pigG1 (Kiknadze et al., 1989).

Correspondingly, the designations of Balbiani rings (BRa, BRb, and BRc) (Figs 2, i-j) in these two species coincide. The inversion in lurG2 sequences covers the entire central part of the arm, including the nucleolus and two Balbiani rings, BRb and BRc (Fig. 2, k). However, the overall banding sequence in *Ch. luridus* arm G is considerably changed due to complex rearrangements in comparison with standard *Ch. piger*.

The Balbiani rings BRb and BRc function in all the cells of salivary glands, whereas the

**Table 3.** Frequencies of genotypic combinations of banding sequences in natural populations of *Chironomus luridus*. \*Keyl’s data (1962), frequencies of genotypic combinations of banding sequences were not determined; presence of a combination is marked by +. N – the number of individuals.

Genotypic combination of banding sequences	Populations							
	West Europe		East Europe	West Siberia, Novosibirsk Province			Kazakhstan, Semipalatinsk nuclear polygon	
	NL-DE-BE N=9	Germany*	RU-YAR-LA N=104	RU-NSK-EL N=50	RU-NSK-KR N=19	RU-NSK-ZI N=8	KZ-SIP-UB N=79	KZ-SIP-SH N=4
lurA1.1	1.000	+	0.923	0.980	1.000	1.000	1.000	1.000
lurA1.2	0	+	0.077	0	0	0	0	0
lurA1.3	0	0	0	0.020	0	0	0	0
lurB1.1	0.778	+	0.942	1.000	1.000	0.875	1.000	1.000
lurB1.2	0	0	0.038	0	0	0	0	0
lurB1.3	0.111	0	0.010	0	0	0	0	0
lurB1.4	0	0	0.010	0	0	0	0	0
lurB1.5	0	0	0	0	0	0.125	0	0
lurB1.6	0.111	0	0	0	0	0	0	0
lurC1.1	0.333	+	0.519	0.920	0.895	1.000	0.797	0.750
lurC2.2	0.334	0	0.087	0	0	0	0	0
lurC1.2	0.333	0	0.394	0.080	0.105	0	0.203	0.250
lurD1.1	1.000	+	1.000	1.000	1.000	1.000	1.000	1.000
lurE1.1	1.000	+	0.990	0.980	1.000	1.000	1.000	1.000
lurE1.2	0	0	0.010	0	0	0	0	0
lurE1.3	0	0	0	0.020	0	0	0	0
lurF1.1	0	+	0	0	0	0	0	0
lurF2.2	0.222	+	0.240	1.000	1.0000	1.0000	1.0000	1.0000
lurF3.3	0.111	0	0	0	0	0	0	0
lurF2a.2a	0	0	0.125	0	0	0	0	0
lurF1.2	0.223	+	0	0	0	0	0	0
lurF2.2a	0	+	0.462	0	0	0	0	0
lurF2.3	0.444	+	0	0	0	0	0	0
lurF2.4	0	0	0.135	0	0	0	0	0
lurF2a.4	0	0	0.038	0	0	0	0	0
lurG1.1	0.778	+	0.999	1.000	1.000	1.000	1.000	1.000
lurG1.2	0.222	0	0.001	0	0	0	0	0

ring BRa develops only in four cells of the salivary gland special lobe (Fig. 2, j), where an additional secretory protein is synthesized (Kiknadze et al., 1989).

The overall pool of banding sequences of the Yaroslavl population appears rather large owing to the chromosomal polymorphism in arms A, B, C, E, F, and G. In total, 16 banding sequences were discovered, which

form 19 genotypic combinations. The level of chromosome polymorphism was also rather high, as up to 80% of individuals in the population were inversion heterozygotes (Table 4). Arms B and F, carrying four and three banding sequences, respectively, appeared to be the most polymorphic.

The specific feature of the Yaroslavl population, as compared with the earlier

studied German populations (Keyl, 1962), is the absence of the sequence lurF1. In addition, we discovered new banding sequences - lurA2, lurB2, lurB3, lurB4, lurC2, lurF4, and lurG2 - in this population.

#### **Western European populations**

Only nine *Ch. luridus* individuals from several water bodies of the Netherlands, Germany, and Belgium were available (Table 1). The main banding sequences in them were identical to those in the population studied in East Europe (Yaroslavl) (Tables 2-3). The western European populations were also similar to the Yaroslavl population in having a high level of chromosome polymorphism (Table 4) and polymorphic arms C, B, F, and G. Their main distinction was the discovery of heterozygotes with the sequence lurF1, undetected in the Yaroslavl population (Tables 2-3; Fig. 3, j). Among the individuals from the Netherlands, lurC2.2 and lurF3.3 homozygotes (Figs 2, d, h, respectively) were detected as well as lurB1.6 heterozygote (Fig. 3, i), which has not been found in other populations. The sequence lurF3 was formed by long simple inversion from lurF2:

lurF2 1a-h 13d-15i 10d-a 16e-a 11a-13c 1i-9f 16f-23f  
 lurF3 1a-h 9f-1i 13c-11a 16a-e 10a-d 15i-13d 16f-23f

#### **Asian populations**

##### *Novosibirsk populations*

In general, the main banding sequences discovered in Novosibirsk populations are identical to the sequences found in the European population (Table 2). However, the Novosibirsk populations are considerably less polymorphic (Tables 2-4). In total, only 11 banding sequences were detected, and the level of heterozygosity in these populations was sevenfold lower. Four arms (A, B, C, and E) were heterozygous versus six arms in the Yaroslavl population. All the heterozygotes detected for these arms were rather rare. The

characteristic features of the Novosibirsk populations studied are a complete domination of lurF2.2 homozygotes and the absence of the wide range of heterozygotes in this arm, which is typical of European populations (Table 3). One larva displayed a unique pericentric inversion covering the centromeric region in chromosome AE (Fig. 3, c), which gave rise to the sequences lurA3 and lurE3. Inverted regions in both sequences, including centromeric band, are distinguished by square brackets

lurA1 1a-2c 4a-9e 2d-3i 12c-10a 13a-19fC 13g-10c 3f-10b 3e-1a lurE1  
 lurA3 1a-2c 4a-9e 2d-3i 12c-10a 13a-19e [13e-g C 19f]  
 lurE1 1a-3e 10b-3f 10c-13g C  
 lurE3 1a-3e 10b-3f 10c-13d [19f C 13g-e].

In addition, the sequence lurB5 (Fig. 3, h) has been detected only in this population. It was not mapped.

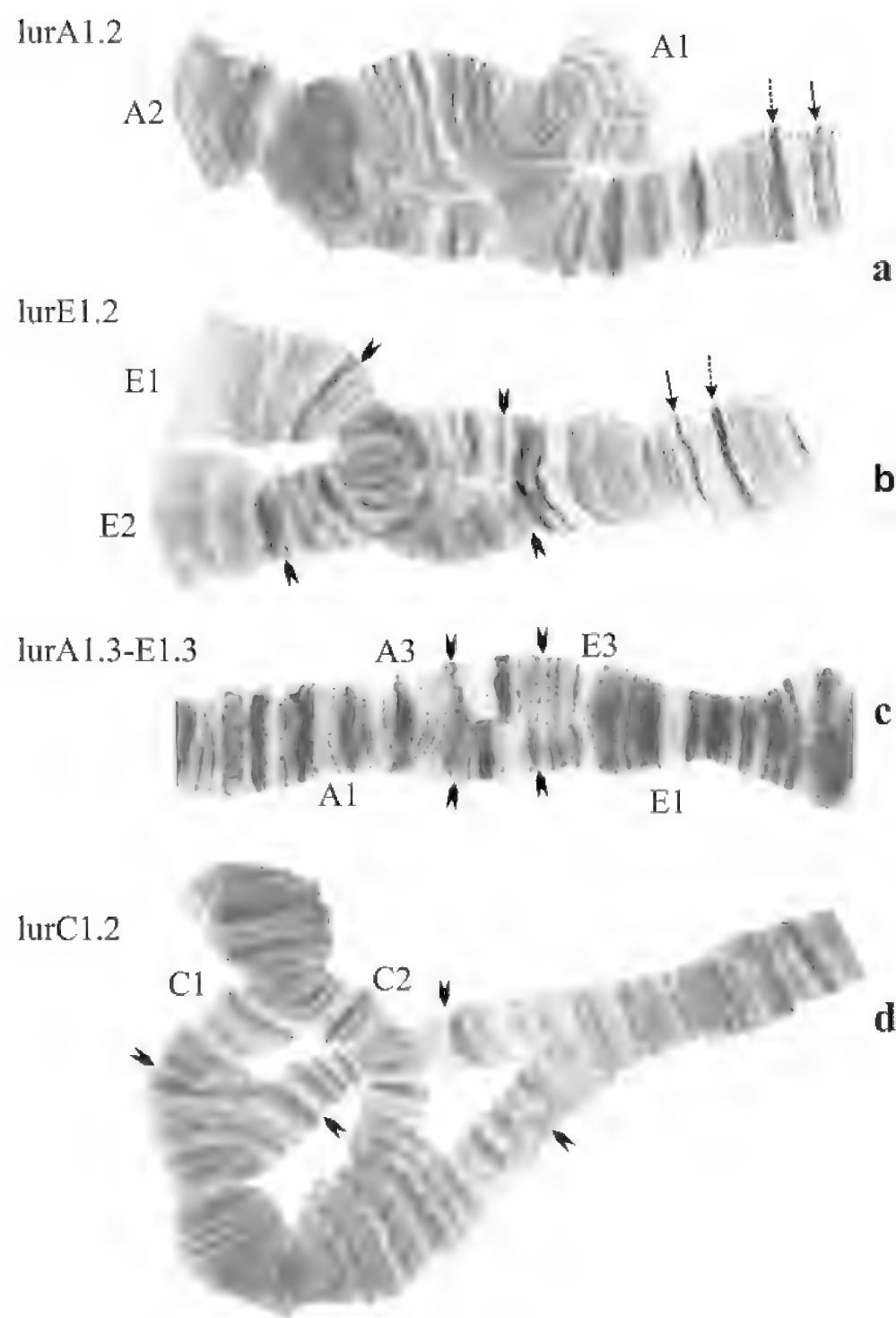
Thus, the Novosibirsk populations are considerably less polymorphic compared with the European populations.

##### *Kazakhstan populations*

These populations were very similar to the Novosibirsk populations in the range and frequencies of inversion banding sequences and in a low level of chromosomal polymorphism (Tables 2-4). Overall, eight banding sequences were detected in these populations.

#### **The centromeric regions**

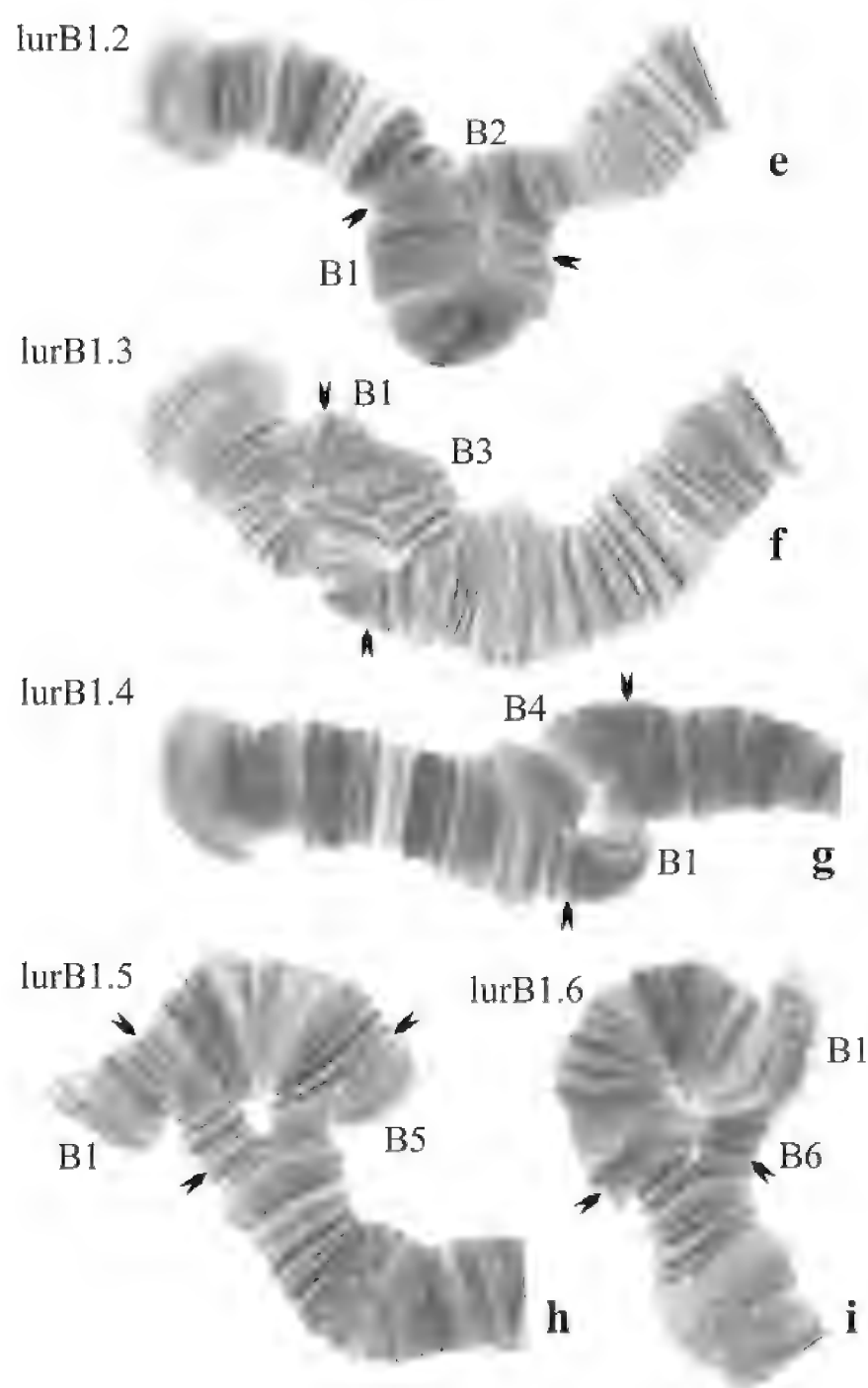
The morphology and molecular characteristics of centromeric bands in the "thummi" cytochrome complex are well studied (Keyl, 1962; Hägele, 1977; Sigareva, 1981; Filippova et al., 1993; Hankeln et al., 1994); however, the data obtained so far for the species of the "pseudothummi" cytochrome complex are insufficient (Keyl, 1962; Kiknadze et al., 1991; Shobanov, Petrova, 1995). Recently, we succeeded in detecting considerable differences in the structure of centromeric regions between the species belonging to the "thummi" and



**Fig. 3, a-d.** Inversion polymorphism in *Ch. luridus*. **a** - heterozygote lurA1.2. **b** - heterozygote lurE1.2. **c** - pericentric inversion in chromosome AE - lurA1.3-E1.3. **d** - heterozygote lurC1.2. Arrowheads indicate the inversion breakpoints. The designations are the same as in Fig. 1.

“pseudothummi” cytocomplexes (Kiknadze et al., 2008b). The translocated chromosomes AE and BF of *Chironomus dorsalis* Meigen, 1818, a member of the “pseudothummi” cytocomplex, appeared dicentric as compared with the untranslocated chromosomes AB and EF in species of “thummi” cytocomplex due to appearance of the centromeric characteristics of bands 19ef in chromosome AE and the bands 28de in chromosome BF. The neocentromeric activity of these bands was suggested.

In this work, we have studied the banding patterns of the centromeric regions in another member of the “pseudothummi” cytocomplex, *Ch. luridus* (Figs 4, a, b). There are no two heterochromatinized bands (centromere and neocentromere) in the translocated chromosomes AE and BF in *Ch. luridus*. The centromeric region of chromosome AE has a very weak centromeric band (Fig. 4, a), bands 19ef are identical with standard. The centromeric region of chromosome BF has also very thin bands 28de (Fig. 4, b), which



**Fig. 3, e-i.** Inversion polymorphism in *Ch. luridus* arm B. **e** - lurB1.2. **f** - lurB1.3. **g** - lurB1.4. **h** - lurB1.5. **i** - lurB1.6. The designations are the same as in Figs. 1 and 3, a-d.

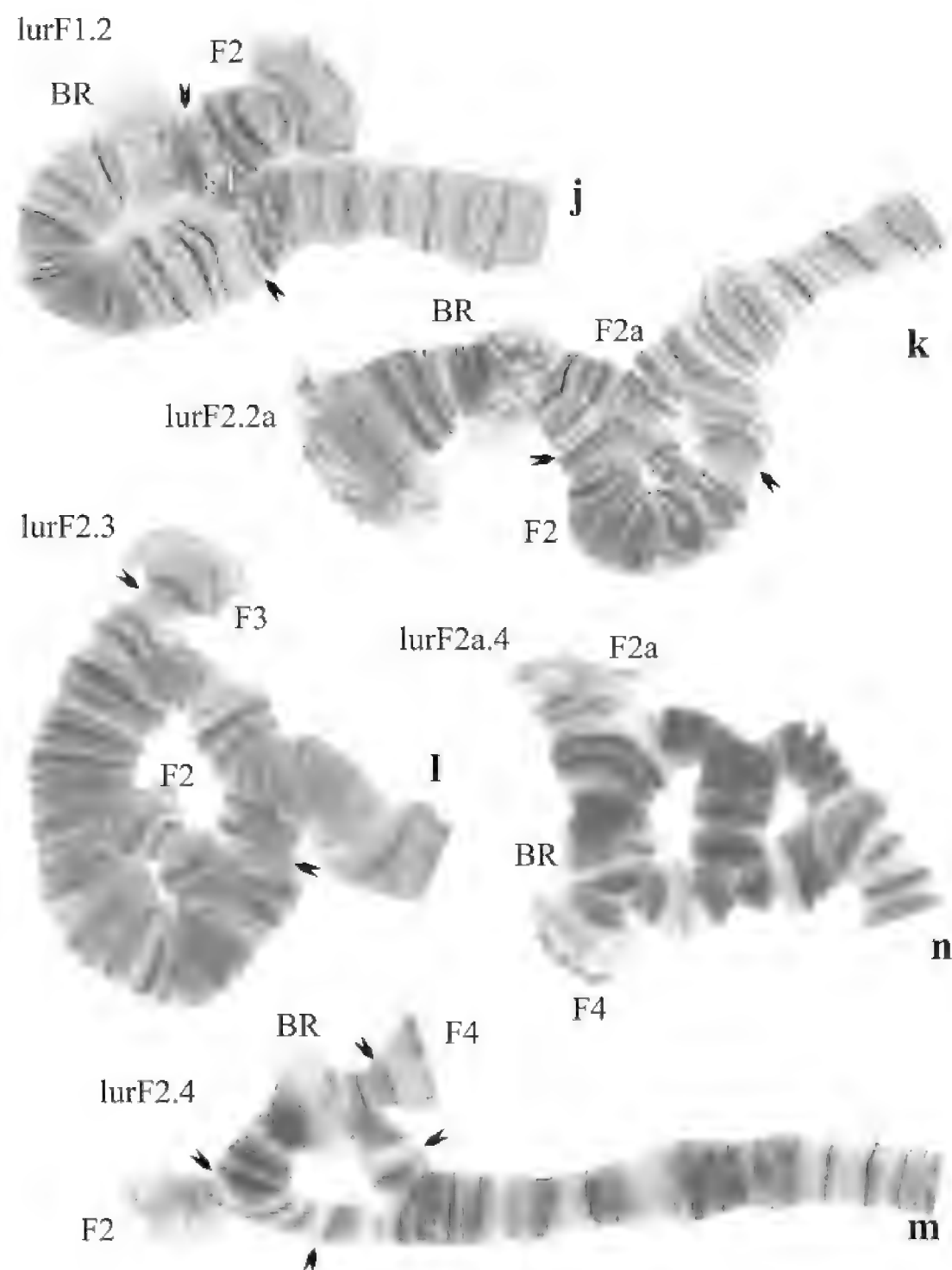
are not heterochromatinized. So, there are no the features of dicentric chromosomes in *Ch. luridus* in comparison with *Ch. dorsalis*. Broshkov (personal communication) has determined only one C-positive band in the translocated chromosomes AE and BF.

### DISCUSSION

In this work, we have analyzed in detail the karyotype, banding patterns, and chromosome polymorphism in *Ch. luridus*.

This study allowed us to estimate the divergence of *Ch. luridus* banding sequences compared with the standard *Ch. piger*, and

to determine the number of chromosome rearrangements that distinguish these karyotypes in five chromosome arms (A, C, D, E, and F), excluding two arms (B and G), where the rearrangements are complex. We have found that arm A of *Ch. luridus* differs by three overlapping inversions, arm E by one simple inversion, arm C by three overlapping inversions, arm D by five overlapping inversions, and arm F by two overlapping inversions from the corresponding arms of *Ch. piger*. Thus, in total, 11 comparatively simple inversions distinguish the *Ch. luridus* and *Ch. piger* karyotypes in five chromosome arms.



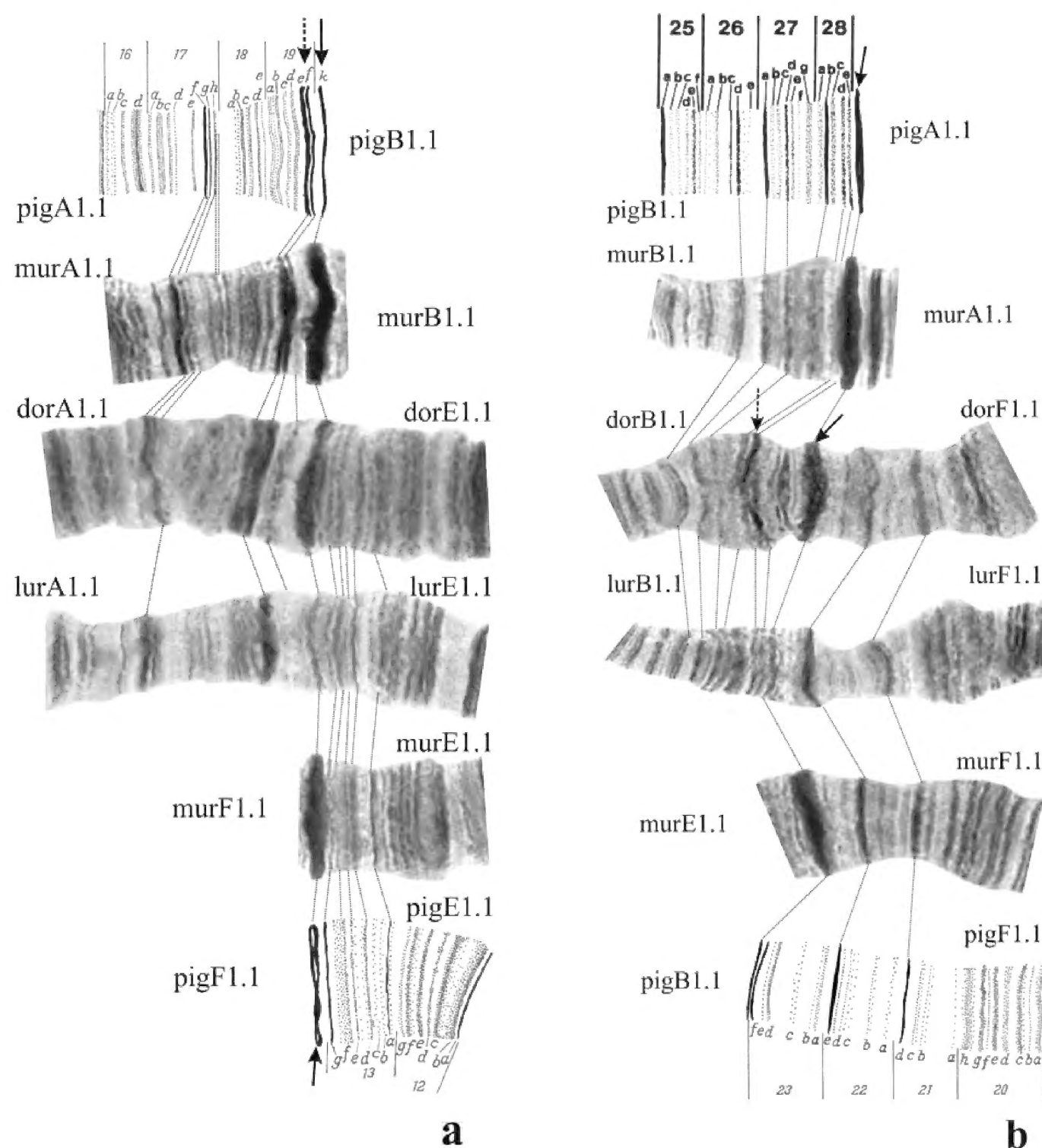
**Fig. 3, j-n.** Inversion polymorphism in *Ch. luridus* arm F. **j** - lurF1.2. **k** - lurF2.2a. **l** - lurF2.3. **m** - lurF2.4. **n** - lurF2a.4. The designations are the same as in Figs. 1 and 3, a-d.

Similar data have been obtained for other species belonging to the “pseudothummi” cytocomplex, namely, 11 inversion steps for *Ch. dorsalis*, 14 for *Ch. pseudothummi* Strenzke, 1959, 18 for *Ch. aprilinus* Meigen, 1938, and 22 for *Ch. uliginosus* Keyl, 1960 (Kiknadze et al, 2008a, b; Broshkov et al., 2008; Kiknadze, Broshkov, 2009; Kiknadze, Istomina, 2009). As a rule, the species of the cytocomplex “thummi” have experienced a larger number of inversions during divergence of their karyotypes from *Ch. piger* (Golygina

et al., 2007).

We have shown that the karyotype of *Ch. luridus* has a high level of chromosomal polymorphism; six of seven chromosome arms (A, B, C, E, F, and G) are polymorphic except arm D. In total, 22 banding sequences form the banding sequences pool of this species.

Comparative study of the chromosomal polymorphism in different *Ch. luridus* populations has demonstrated significant differences among the populations in the sets and frequencies of inversion banding



**Fig. 4, a, b.** Mapping of the centromeric regions in chromosomes AE (**a**) and BF (**b**) of *Chironomus luridus*, *Ch. piger*, and *Ch. muratensis* Ryser, Scholl, Wülker, 1983. Solid arrows show the centromeric bands, and dashed arrows indicate bands 19ef on arm A (**a**) and 28de on arm B (**b**), which demonstrate neocentromeric characters in *Ch. dorsalis*

sequences. The most polymorphic of the populations studied was the Yaroslavl population, which contained 16 banding sequences and six polymorphic chromosome arms. The overall level of polymorphism in this population was also high, as almost 80% of individuals in the population were inversion heterozygotes. The populations from Western Europe were similar to the Yaroslavl population in having a high level of polymorphism.

On the contrary, the Siberian and

Kazakhstan populations display considerably smaller sets and lower frequencies of inversion sequences, as well as a lower total level of inversion heterozygosity. In these populations mainly arm C was polymorphic, but very rare heterozygotes in arm B were also recorded. Among the most pronounced distinctions between the European, Siberian, and Kazakhstan populations was a drastic decrease in the sets and frequencies of inversion polymorphism in arm F. The studied Western

**Table 4.** Inversion polymorphism in *Chironomus luridus* populations. N – the number of individuals.

Inversion polymorphism in populations.	Populations						
	West Europe	East Europe	West Siberia, Novosibirsk Province			Kazakhstan, Semipalatinsk nuclear polygon	
	NL-DE-BE N=9	RU-YAR-LA N=104	RU-NSK-EL N=50	RU-NSK-KR N=19	RU-NSK-ZI N=8	KZ-SIP-UB N=79	KZ-SIP-SH N=4
Heterozygous larvae, %	77.8	79.8	12	10.5	12.5	20.2	25.0
Average number of heterozygous inversions per larvae	1.333	1.17	0.140	0.10	0.125	0.2	0.25
Number of banding sequences per population	13	16	10	8	8	8	7
Number of genotypic combinations per population	15	19	10	8	8	8	7

and Eastern European populations displayed four sequences (lurF2, lurF2a, lurF3, and lurF4) at a rather high frequency in various genotypic combinations (lurF2.2, lurF2a.2a, lurF2.2a, lurF2.3, and lurF2a.4) versus the Novosibirsk and Kazakhstan populations, where arm F was monomorphic with only the sequence lurF2.

The sequence lurF1, described by Keyl (1962), was not detected in any of the studied populations except for the population from the Netherlands. Keyl noted that the four German populations that he studied displayed differences connected with the presence of lurF1 and lurF2; unfortunately, he did not mentioned whether these differences were connected with the occurrence of homozygotes at lurF1.1 and lurF2.2 or only with lurF1.2 heterozygotes. Strangely, Keyl did not give any photograph of either homozygote lurF1.1 or homozygote lurF2.2, which considerably hinders the comparison of the polymorphism in Western European populations with Eastern European, Siberian, and Kazakhstan populations. However, the fact that the

difference connected with the inversion polymorphism of *Ch. luridus* populations is essentially associated with the polymorphism in arm F is undoubted.

In spite of some differences in banding sequences between populations we view these differences as falling within the range of intraspecies polymorphism as well in many other *Chironomus* species (Gunderina, Kiknadze, 1999; Kiknadze, 2008).

According to Dobzhansky (1970), central populations of a species are the most polymorphic. So, it is possible to suggest that European populations of *Ch. luridus* can be considered as central while Asian populations as peripheral.

The karyotypes of the species belonging to the “pseudothummi” cytocomplex differ from the karyotypes of the species belonging to the “thummi” cytocomplex by the presence of reciprocal whole-arm translocations in two chromosomes: AE and BF in the “pseudothummi” cytocomplex have become AB and EF in the “thummi” cytocomplex.

The specific feature of the *Ch. luridus*

karyotype, similar to the karyotypes of some other “pseudothummi” cytocomplex species (*Ch. pseudothummi*, *Ch. uliginosus*, and *Ch. aprilinus*), is the change in the centromeric region structure of translocated chromosomes AE and BF compared with the species of the “thummi” cytocomplex (Kiknadze et al., 2008a; Broshkov et al., 2008; Kiknadze, Broshkov, 2009; Kiknadze, Istomina, 2009) The centromeric bands in chromosomes AE and BF of the species of the “pseudothummi” cytocomplex investigated are very thin, and it is difficult to determine their presence, but they are not dicentric as in *Ch. dorsalis*.

#### ACKNOWLEDGEMENTS

The authors are grateful to Dr. Henk Vallenduuk for his kind provision of *Ch. luridus* from the Netherlands. The work was supported by the Presidium of the Russian Academy of Sciences under the programs “The Origin of Biosphere and Evolution of Geo-biological Systems”, and “Biodiversity”.

#### REFERENCES

- Acton A.B. 1957.** Chromosome inversions in natural populations of *Chironomus dorsalis* // *J. Genetics*. 55: 261-275.
- Ayala F.J., Coluzzi M. 2005.** Chromosome speciation: human, *Drosophila*, and mosquitoes // *PNAS*. 102(1): 6535-6542.
- Belyanina S.I. 1983.** The karyotypic analysis of the non-biting midges (Chironomidae, Diptera) of the USSR fauna. Dr. Sci. Dissertation, A.N. Severtsov Institute of evolutionary morphology and ecology of animals, Russian Academy of Sciences. Moscow. 455 p. (In Russian).
- Broshkov A.D., Kiknadze I.I., Istomina A.G., Gunderina L.I. 2008.** The karyotype structure of the midge *Chironomus uliginosus* Keyl, 1960 (Diptera, Chironomidae) // *Euroasian Entomol. J.* 7(1): 57-65. (In Russian with English summary).
- Dévai Gy., Miskolczi M., Wülker W. 1989.** Standardization of chromosome arms B, C and D in *Chironomus* (Diptera, Chironomidae) // *Acta Biol. Debr. Oecol. Hungarica*. 2(1): 79-92.
- Dobzhansky Th. 1970.** Genetics of the evolutionary process. New York. 505 p.
- Filippova M.A., Kiknadze I.I., Aimanova K.G., Fischer J., Blinov A.G. 1993.** Homology of Balbiani rings among chironomid species and localization of a new mobile element on the polytene chromosomes // *Netherlands J. Aquat. Ecology*. 26(2-4): 123-128.
- Golygina V.V., Kiknadze I.I., Istomina A.G., Gunderina L.I., Miroshnichenko L.A., Gusev V.D. 2007.** Cytogenetic divergence of genomes in *Chironomus plumosus* group (Diptera: Chironomidae) // *Comp. Cytogenet.* 1(1): 17-32.
- Gunderina L.I., Kiknadze I.I. 1999.** Interspecific cytogenetic differentiation of the sibling species *Chironomus plumosus* L. and *Chironomus balatonicus* Devai, Wulker, Scholl (Chironomidae: Diptera) // *Russ. J. Genet.* 35(9): 1023-1029.
- Hägele K. 1977.** Differential staining of polytene chromosome bands in *Chironomus* by Giemsa banding method // *Chromosoma*. 59(3): 207-216.
- Hankeln T., Filippova M.A., Kiknadze I.I., Aimanova K.G., Schmidt E.R. 1994.** Centromeric heterochromatin and satellite DNA in the *Chironomus plumosus* species group // *Genome*. 37: 925-934.
- Keyl H.-G. 1962.** Chromosomen evolution bei *Chironomus*. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten // *Chromosoma*. 13: 464-514.
- Keyl H.-G., Keyl I. 1959.** Die cytologische Diagnostik der Chironomiden. I. Bestimmungstabelle für die Gattung *Chironomus* auf Grund der Speicheldrusenchromosomen // *Arch. Hydrobiol.* 56(1/2): 43-57.
- Kiknadze I.I. 2008.** The role of chromosomal polymorphism in divergence of populations and species of the genus *Chironomus* // *Zool. Zh.* 87(6): 686-701 (In Russian with English translation in *Entomol. Review*. 2008. 88(5): 509-524).
- Kiknadze I.I., Blinov A.G., Kolesnikov N.N. 1989.** The molecular-cytological organization of chironomid genome, (pp. 4-58) // Shumny V.K. (Ed.) *Structural and functional organization of genome*. Novosibirsk, 189 p. (In Russian with English summary).
- Kiknadze I.I., Broshkov A.D. 2009.** The karyotype and chromosomal polymorphism of the midge *Chironomus melanescens* Keyl, 1961 (Diptera, Chironomidae) // *Euroasian Entomol. J.* 8. Suppl.1: 19-28. (In Russian with English summary).

- Kiknadze I.I., Broshkov A.D., Istomina A.G., Gunderina L.I., Vallenduuk H. 2008a.** The geographic variability of the polytene chromosome banding sequence of non-biting midge *Chironomus pseudothummi* Str. (Diptera, Chironomidae) // *Tsitologiya*, 50 (6): 539-548. (In Russian with English translation in *Cell and Tissue Biology*. 2(4): 417-427).
- Kiknadze I.I., Golygina V.V., Broshkov A.D., Gunderina L.I., Istomina A.G. 2008b.** Mystery of *Chironomus dorsalis* Meigen karyotype (Diptera, Chironomidae) // *Comp. Cytogenet.* 2(1): 21-35.
- Kiknadze I.I., Grebenyuk L.P., Filippova M.A., Kerkis I.E. 1988.** Karyological analysis of *Chironomus* species of *pseudothummi* cytocomplex from USSR water bodies // *Biol. Interland Waters Inform. Bull.* 79: 56-59. (In Russian).
- Kiknadze I.I., Gunderina L.I., Istomina A.G., Gusev V.D., Nemytikova L.A. 2003.** Similarity analysis of inversion banding sequences in chromosomes of *Chironomus* species (breakpoint phylogeny) (pp. 245-253) // Kolchanov N., Hofstaedt R. (Eds.). *Bioinformatics of Genome Regulation and Structure*. Boston, Dordrecht, London. 373 p.
- Kiknadze I.I., Istomina A.G. 2009.** The chromosomes variability in natural populations of midge *Chironomus aprilius* Meigen (Diptera, Chironomidae) // *Euroasian Entomol. J.* 8: 9-17. (In Russian with English summary).
- Kiknadze, I.I., Shilova, A.I., Kerkis, I.E., Shobanov, N.A., Zelentsov, N.I., Grebenyuk, L.P., Istomina, A.G. & Prasolov, V.A. 1991.** Karyotypes and larval morphology in tribe Chironomini. Atlas. Novosibirsk. 115 p. (In Russian with English summary).
- King M. 1993.** Species Evolution: the Role of Chromosomal Change. New York. 336 p.
- Martin J. 1979.** Chromosome as tools in taxonomy and phylogeny of Chironomidae (Diptera) // *Entomol. Scandinavica*. (Supplement). 10: 67-74.
- Martin J. 2007.** North American cytospecies of the genus *Chironomus* // <http://www.genetics.unimelb.edu.au/Martin/NACytfiles/NACytospecies07.pdf>, on 28 October 2009.
- Michailova P.V. 1989.** The polytene chromosomes and their significance to the systematics of the family Chironomidae, Diptera // *Acta Zool. Fennica*. 186: 1-107.
- Shobanov N.A., Petrova N.A. 1995.** Karyotype peculiarities in *Chironomus saxatilis* Wülker et al., 1981 (Diptera, Chironomidae) from the Siberian arctic region and a possible origin of neocentromeres // *Tsitologiya*. 37(7): 585-591. (In Russian with English summary).
- Sigareva L.E. 1981.** The differential staining of chromosomes in chironomid // *Tsitologiya*. 23(3): 270-274. (In Russian with English summary).
- White M.J.D. 1973.** Animal cytology and evolution. Cambridge. 961 p.
- Wülker W. 1980.** Basic patterns in the chromosome evolution of the genus *Chironomus* (Diptera) // *Z. Zool. Syst. Evolutionsforsch.* 18: 112-123.
- Wülker W. 2007.** The role of chromosomes in chironomid systematics, ecology and phylogeny // <http://insects.ummz.lsa.umich.edu/~ethanbr/chiro/Docs/Wuelker/polytene.html>, on 28 October 2009.
- Zdobnov E.V., von Mering C., Letunic I., Torrents I., Suyama M., Copley R.R., Christophides G.K., Thomasova D., Holt R.A., Mani Subramanian G., Mueller H.-M., Dimopoulos G., Law J.H., Wells M.A., Birney E., Charlab R., Halpern A.L., Kokoza E., Kraft C.L., Lai Z., Lewis S., Louis C., Barillas-Mury C., Nusskern D., Rubin G.M., Salzberg S.L., Sutton G.G., Topalis P., Wides R., Wincker P., Yandell M., Collins F.H., Ribeiro J., Gelbart W.M., Kafatos F.C., Bork P. 2002.** Comparative genome and proteome analysis of *Anopheles gambiae* and *Drosophila melanogaster* // *Science*. 298: 149-159.

Received October 28, 2009.

Accepted by S. Grozeva, February 11, 2010.

Published July 09, 2010.

